

# THE OHIO JOURNAL OF SCIENCE

---

VOL. XXXVII

MARCH, 1937

No. 2

---

## SOME CYTOLOGICAL OBSERVATIONS ON PERIPHERAL MYELINATED NERVE FIBERS AS OBSERVED BY THE POLARIZED LIGHT METHOD

H. E. SETTERFIELD AND H. M. WEAVER,  
Department of Anatomy, The Ohio State University

### INTRODUCTION

Most neurological procedures depend upon the constancy of the artefacts produced by fixation and staining. Many observations have been made on living or freshly killed tissues with limited results. George St. de Renyi ('29), in particular, has studied nerve fibers by the microdissection method in an attempt to determine the actual cytological characteristics of the fiber while its protoplasm is in a living condition. His findings are of the greatest interest although his negative results in regard to certain structures leave the problem open to further investigation. In a previous paper of one of us (Setterfield and Sutton, '35) it was pointed out that we believe we approximate the observation of unaltered protoplasm by using the polarized light method. This technique has tended to demonstrate the existence in vivo of many of the cytological features which have been described by means of staining methods. It is our purpose in this paper to describe some of these structures as seen in polarized light and compare them with the same structures as they are usually described and illustrated in standard text books on the subject, notably Ramon y Cajal ('28) in his "Degeneration and Regeneration of the Nervous System," Volume one.

### MATERIALS AND METHOD

The materials for this study are nerves from the brachial plexus, lumbo-sacral plexus and nerves from the lumbar area, from man, monkeys, dogs, cats, and rats suffering from various pathological

conditions. Normal nerves used for comparison were removed from healthy rats, cats, dogs, and monkeys. The source of the nerve trunk for each submitted photomicrograph is identified in its accompanying legend.

As the polarized light technique has been discussed in the paper previously cited, it is useless to do so here.

#### DISCUSSION

It may be objected that the structures described are not normal but the result of pathological changes. It is true that some of the structures observed are almost or quite invisible in the normal nerve fiber in polarized light; but this is equally true of many structures described by Cajal and others, as their cases were usually from animals following operative procedures and occurring in early stages of degeneration. In other words, our purpose is not to determine the normality of the structures in question but to try to identify them as artefacts or actual structures in the living nerve fiber. Furthermore, the study of nerve trunks removed from patients or from experimental animals suffering from pathological conditions secondarily affecting the peripheral nervous system is well adapted to this type of cytological study as the changes produced are more gradual and much less intensive. Also, since the nerve trunks have not been operatively cut the fibers remain in their own sheaths, thus eliminating the possibility of entrance of foreign material or the escape of protoplasm normally found in the fiber.

As has been pointed out in the paper cited above, swelling of the fibers with seemingly great increase in fluid content is the first and most striking early manifestation of disease in nerve fibers. This type of pathological change is essentially similar to cloudy swelling noted in all other portions of the body suffering from disease. So far as we have been able to determine this swelling does not alter the optical properties of the various parts of the fiber, merely serving to more clearly delimit details due to their greater size and separation from surrounding structures.

Attention was directed in another paper (Setterfield and Baird, '36) to the fact that the axis cylinder, sheath of Schwann, cementing disk, neurokeratin, and the incisures of Schmidt-Lantermann are isotropic in polarized light while the myelin itself is the only birefringent structure in the nerve fiber. We must now point out further that there are differences in the appearance of some of these structures when carefully examined. Specifically, the true axis cylinder (see below), in polarized light, is deep black at all points in the rotation of the stage of the microscope. On the other hand, the cementing disk, though isotropic (that is, not changing from black to white with rotation of the stage) is not as intense a black as the true axis cylinder. Similarly, the non-neurofibrillated portion of the axis cylinder (see below) is slightly less intensely black than the cementing disk, though unchanging during the rotation of the stage. In addition to these structures, still others show less intensities of blackness in the following order: neurokeratin (slightly degenerated), perineurium, bracelets of Nageotte, and the

sheath of Schwann. The perineurium is a brownish-gray at all points of stage rotation; the bracelets of Nageotte a medium gray, and the sheath of Schwann a pale gray. These intensities of blackness and whiteness are constant for the structure and are all indicative of isotropism which is manifested by the failure to change from black to white during the rotation of the stage. On the other hand, the myelin sheath, as the stage is rotated, changes from deep black to white four times in each revolution. It is the only anisotropic substance in the fiber. All the remainder are isotropic, but with varying degrees of blackness and whiteness to the eye and to the photographic plate.

In considering the nerve trunk as a whole it might be said in passing that the epineurium appears to be birefringent. Due to the contraction of its connective tissue fibers after death this birefringent appearance might be due to an optical phenomenon resulting from the passing of polarized light through this wavy tissue. Since it is not nervous tissue, it has not been the object of extended study.

Cajal ('28) stated that fixation of nerve trunks in formalin resulted in a shrinkage of the myelin sheath. An extended study was made by one of us (H. M. W.) on this phase of the problem. Nerves removed at cancellation were divided and one-half immediately frozen, sectioned and observed. The remaining half of the nerve trunk was allowed to remain in a fifteen per cent. aqueous solution of formalin for variable lengths of time, up to six weeks. Although it was impossible to obtain actual measurements it was felt that no shrinkage had occurred while in formalin. Due to the necessity of making thin sections the unfixed tissue so badly crumbled as to make photographic recording unsatisfactory.

Two semi-diagrammatic drawings have been included in this paper presenting in visual form the writers' conception of two physiological phases of nerve fibers of the peripheral nervous system. These drawings do not show exact quantitative differences in blackness and whiteness but have been exaggerated for purposes of illustration.

#### OBSERVATIONS

Bracelets of Nageotte (Fig. 1 and Drawing 1 and 2), as viewed in polarized light, seem to be paired cup-like structures with their bases approximating the cementing disk and their sides extending outward into the myelin sheath and a little nearer the sheath of Schwann than the axis cylinder. As to the origin of these structures the writers could not be sure, although it is possible that they represent an inward and upward reflection of the sheath of Schwann, at the level of the cementing disk. Most standard texts describe these structures as a ring of spines projecting away from the cementing disk. It would seem to the writers that this appearance might be due to either the wrinkling of this structure and the subsequent heavy deposition of staining material in the grooves, or to the fact that the nerve fiber has been cut in such a way that the top of the "cup" has been removed. Due to the diagonal cutting of a nerve fiber the bracelets here reproduced in Figure 1 show both these conditions.

The cementing disk (Fig. 2 and Drawings 1 and 2) is another

structure that is easily seen in polarized light, appearing as a transversely located plate into whose periphery runs the sheath of Schwann.

The sheath of Schwann (Fig. 6 and Drawings 1 and 2) has the same structural appearance in polarized light as in stained preparations. It is extremely thin, pale gray in color, and thickened slightly at the location of the nucleus of the Schwann cell and as the sheath approaches the node of Ranvier. Spherules of Erlholz could not be observed in the cytoplasm of the Schwann cell. Also the "dust" particles described by Swank and Davenport ('34) were invisible; indicating that the spherules of Erlholz and the "dust" particles do not exhibit the same optical properties as degenerating myelin, although their reaction to osmic acid is the same. The endocellular apparatus of Golgi likewise could not be seen. There are five possibilities regarding the relation of the sheath of Schwann with the cementing disk, none of which could be accurately determined with the neurological method under discussion.

1. The sheath of Schwann may be continuous over each cementing disk.
2. The sheath of Schwann may be contiguous with the substance of the cementing disk.
3. The sheath of Schwann may be reflected backwards, at the cementing disk, over the axis cylinder to form the sheath of Mauthner.
4. The sheath of Schwann may be reflected backwards, at the cementing disk, to form the bracelet of Nageotte.
5. The cementing disk may be formed by the fusing of the sheaths of Schwann from two adjacent internodal segments.

Due to our inability to differentiate the ending of the sheath of Schwann and the beginning of the cementing disk, and to a series of isolated observations during various stages of nerve fiber degeneration, the writers are inclined to favor the fifth hypothesis.

The sheath of Mauthner could not be identified in any fiber we have ever observed.

Myelin, in a normal nerve fiber (Fig. 2 and Drawings 1 and 2), is homogeneous and birefringent. As degeneration phenomena in the nerve fiber progress the myelin (Fig. 4) rapidly becomes isotropic as it changes into its degeneration products.

Neurokeratin (Fig. 3 and Drawings 1 and 2), in the normal nerve fiber, has an index of refraction which is only slightly different from the myelin. It seems, however, that its presence can be traced through the myelin, under oil immersion, as a thin, skeletal system of trabeculae. Therefore the writers feel that, in the normal condition, the very slightly granular appearance of the myelin is due to the presence of the neurokeratin framework. In rather early degenerating fibers this material swells and rapidly becomes black, (as seen in polarized light), thereby facilitating identification.

In the normal condition the axis cylinder (Drawing 2), as commonly referred to in the literature, is seen to be composed of two portions, (1) an inner cylindrical rod and (2) an outer enveloping sheath. The inner cylindrical rod, which we have called the true axis cylinder, is, in polarized light, isotropic, deep black, homogeneous, and of a uniform

calibre throughout the length of the nerve fiber. The outer enveloping sheath which Maximow ('34) calls the non-neurofibrillated portion of the axis cylinder is, in polarized light, isotropic but less intensely black than the true axis cylinder, thereby facilitating its identification. Practically every author has described the axis cylinder to be of constant calibre except at two points; an enlargement above and below, and a constriction as it passes through, the cementing disk. By reference to the included drawings it can be seen that it is the non-neurofibrillated portion of the axis cylinder only that is involved in this enlargement and constriction. It is generally assumed that the non-neurofibrillated portion of the axis cylinder is co-extensive with the true portion. In view of our observations on its relation to neurokeratin we have suspected that the non-neurofibrillated portion is related to the myelin segment rather than the true axis cylinder. While we cannot demonstrate conclusively that the non-fibrillated portion is interrupted at the cementing disc, our observations have cast doubt on the assumption of its continuity. At present we are unable to make a definite statement on the question.

In one physiological phase of the normal nerve fiber (Drawing 2) the incisures of Schmidt-Lantermann are extremely narrow with an index of refraction so nearly the same as myelin as to render them almost invisible.

In another phase the relation of the true axis cylinder, the non-neurofibrillated portion of the axis cylinder, and the incisures of Schmidt-Lantermann, is extensively altered. In this phase (Figs. 5, 6, 8 and Drawing 1) it can be seen that the true axis cylinder is, to all appearances, identical with that shown in Drawing 2 with the exception that there are areas which lie in direct contact with material which appears to be myelin. That this portion of the axis cylinder is the functional, and hence the true portion, has been clearly shown in work on anterior poliomyelitis, to be reported on in the future. Whenever fragmentation of the true axis cylinder was observed the animal always exhibited signs of paralysis.

One of the most interesting and, as yet, unexplained conditions in both early degenerating and physiological states of nerve fibers, is that the non-neurofibrillated portion of the axis cylinder seems to split at the level of the innermost part of an incisure of Schmidt-Lantermann. After this splitting it, to all appearances, slides upward and outward over the incisure of Schmidt-Lantermann until it comes into contact with the sheath of Schwann. That this sliding upward involves all of the non-neurofibrillated portion of the axis cylinder from one incisure to another is evidenced by a short area in which the true axis cylinder is in direct contact with myelin. By reference to the drawing it can be seen clearly that the so-called incisures of Schmidt-Lantermann (Figs. 5, 8, and Drawing 1), (which, in reality, are composed of an upward and outward extension of the non-neurofibrillated portion of the axis cylinder over the original incisural structure) together with their inferior prolongations (the non-neurofibrillated portion of the axis cylinder) form a true infundibulum ("funnel"). Through the tube and dilated mouth of the infundibulum is found extending the true axis

cylinder. The true axis cylinder can easily be likened to a stream of fluid running through several superimposed funnels.

The presence of neurofibrils is not doubted by most histologists. However, de Renyi ('29) reached the conclusion that there are no neurofibrils in the living nerve fiber of vertebrates he studied. Observations with polarized light have not settled this question to the satisfaction of the writers. In one notable example, illustrated in Figure 7, in a case twelve hours after cutting the nerve, structures were seen which one of us (H. E. S.) firmly believes to be neurofibrils. Up to the present time this is the only nerve studied at that particular length of time after operation and it may be that it is only at this or corresponding stage of degeneration that they are made visible by the swelling within the true axis cylinder and are not yet degenerated to the extent that their identity is lost. In all other cases, both normal and pathological, the true axis cylinder has shown a homogeneous structure throughout.

In a few cases we have been able to see axonal rings (Fig. 8) similar to those described by Cajal. They appear to be condensations of neurokeratin on the surface of the non-neurofibrillated portion of the axis cylinder, never on the true axis cylinder as above defined. They are quite variable in appearance and we do not believe they are present in normal fibers.

The authors wish to call attention in particular to the relation of the neurokeratin framework to the infundibulum and to the myelin. It appears that the neurokeratin, from its greatest concentration immediately surrounding the infundibulum (non-neurofibrillated portion of axis cylinder in some phases of nerve fibers), extends outward through the myelin to reach its minimum of concentration at its junction with the sheath of Schwann into which it seems to blend. This spongy neurokeratin framework contains potential cavities of varying size; potential because they are filled with true myelin.

#### CONCLUSIONS

1. Polarized light as a neurological technique depends upon the chemical structure and consequent optical properties of the materials observed. Certain lipoids, making up the substance of the myelin, have the property of birefringence while the chemical complex characterizing the other structures of the nerve fiber do not. This, together with a difference in the degree of isotropism of the non-birefringent structures, makes a study of individual structures both easy and accurate.

2. In this technique we believe that we possess a more sensitive, much simpler and more rapid method than any other now in use for the study of myelinated nerve fibers.

3. We have been able to describe in some detail the structure of nerve fibers, in so far as cytological characteristics are concerned. Our observations have tended to verify the existence

in vivo of several structures which some have suspected of being artefacts of staining and to deny the actuality of others described as characteristically present in nerve fibers.

4. Polarized light technique demonstrates the actuality in unstained nerve trunks of the sheath of Schwann, and its nucleus, perineurium, epineurium, myelin, neurokeratin, incisure of Schmidt-Lantermann, infundibulum, true axis cylinder and possibly its neurofibrils, non-neurofibrillar portion of the axis cylinder, cementing disk, bracelet of Nageotte, fissural, and axonal rings.

5. The polarized light method, according to our study, does not demonstrate the following structures: extra-fissural rings, sheath of Mauthner, spherules of Erlholz, "dust" particles, endocellular apparatus of Golgi, and apparatus of Golgi-Rezzonico.

6. We believe that the differences in intensities of blackness and whiteness of the various isotropic structures are due to a difference in thickness, chemical complex, or to a combination of both.

#### ACKNOWLEDGMENTS

The writers wish to express their appreciation for the co-operation of the following persons:

1. Dr. P. C. Kitchin, of the Department of Dental Research of the College of Dentistry, for the use of the polarizing microscope and photographic equipment.

2. Drs. H. B. Davidson and T. T. Frost, of the Department of Pathology of the College of Medicine, for furnishing the human nerves used in this study.

3. Dr. J. A. Toomey, of the Cleveland City Hospital, Cleveland, Ohio, for permitting us to use the photomicrograph of a nerve from a monkey suffering from anterior poliomyelitis.

4. Miss Elizabeth Mitchell, for making the drawings used in this paper.

#### BIBLIOGRAPHY

- Cajal, Ramon y. 1928. Degeneration and Regeneration of the Nervous System. Oxford University Press, London, Vol. 1.
- Maximow, Alexander A. and Bloom, William. 1934. Textbook of Histology. W. B. Saunders Company, Philadelphia.
- Renyi, George St. de. 1929. The Structure of Cells as revealed by Microdissection. II. The Physical Properties of the Living Axis Cylinder in the Myelinated Nerve Fiber of the Frog. *Journal of Comparative Neurology*, Vol. 47, No. 3, pp. 405-425.
1929. The Structure of Cells as revealed by Microdissection. III. Observations on the Sheaths of Myelinated Nerve Fibers of the Frog. *Journal of Comparative Neurology*, Vol. 48, No. 2, pp. 293-310.
1929. The Structure of Cells as revealed by Microdissection. IV. Observations on Neurofibrils in the Living Nervous Tissue of the Lobster (*Homarus americanus*). *Journal of Comparative Neurology*, Vol. 48, No. 3, pp. 441-457.

- Setterfield, H. E. and Baird, T. T. 1936. The Polarized Light Technique: A Comparison with the Marchi Method. *Stain Technology*, Vol. 11, No. 2, pp. 41-44.
- Setterfield, H. E. and Sutton, T. S. 1935. Use of Polarized Light in the study of Myelin Degeneration. I. The Appearance and Progress of Degeneration after Transection of the Sciatic Nerve of the White Rat. *Anatomical Record*, Vol. 61, No. 4, and Supplement, pp. 397-411.
- Swank, R. L. and H. A. Davenport. 1934. The Marchi Staining Method: Fixation. *Stain Technology*, Vol. 9, No. 4, p. 129.

## EXPLANATION OF PLATES

### DESCRIPTION OF PLATE I

- Fig. 1. Median nerve of human removed at autopsy. Anatomical Diagnosis: attempted death by hanging; patient remained alive twenty-eight hours without regaining consciousness; edema and severe hyperemia of the lungs and brain.  $\times 1000$ .
- Fig. 2. Median nerve of young monkey removed under ether anesthesia. Anatomical Diagnosis: Caseous tuberculosis of lower left lung.  $\times 1800$ .
- Fig. 3. Median nerve of human removed at autopsy. Anatomical Diagnosis: right ulcerative pulmonary tuberculosis; left caseous pulmonary tuberculosis.  $\times 1000$ .
- Figs. 4 and 5. Median nerve of human removed at autopsy. Anatomical Diagnosis: multiple infarcts of spleen, kidneys, and lungs; acute hemorrhagic gastritis; toxic splenomegalia; etc.  $\times 1000$ .
- Fig. 6. Sciatic nerve of cat removed under ether anesthesia following experimental starvation for two weeks. Anatomical Diagnosis: negative.  $\times 1800$ .
- Fig. 7. Peripheral stump of sciatic nerve of an albino rat, twelve hours following surgical section.  $\times 1000$ .
- Fig. 8. Sciatic nerve of a young monkey showing paralysis of the leg for two days following experimentally produced anterior poliomyelitis.  $\times 1000$ .

### EXPLANATION OF FIGURES 1 AND 2

Semi-diagrammatic drawings of two different physiological phases of a normal nerve fiber from the peripheral nervous system.

1. Non-neurofibrillated portion of the axis cylinder.
2. Sheath of Schwann.
3. True myelin.
4. Naked axis cylinder.
5. Infundibulum.
6. Neurokeratin trabecula.
7. Swollen part of non-neurofibrillated portion of axis cylinder.
8. Cementing disk.
9. Bracelet of Nageotte.
10. True axis cylinder.
11. Ostium of Infundibulum.
12. Nucleus of Schwann cell.
13. Incisure of Schmidt-Lantermann.
14. Infundibular ring.





